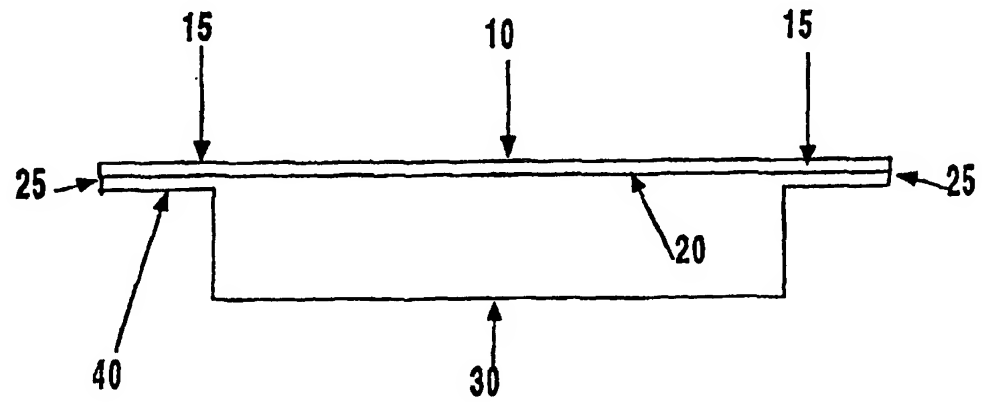


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<p>(21) International Application Number: PCT/US96/06334 (22) International Filing Date: 6 May 1996 (06.05.96) (30) Priority Data: 08/486,979 7 June 1995 (07.06.95) US (71) Applicant: CLARION PHARMACEUTICALS INC. [US/US]; 585 Science Drive, Madison, WI 53711 (US). (72) Inventors: PRUSS, Thaddeus, P.; 2972 Woods Edge Way, Madison, WI 53711 (US). WILL, James, A.; 344 South Charles Street, Columbus, WI 53925 (US). (74) Agents: ZELANO, Anthony, J. et al.; Millen, White, Zelano & Branigan, P.C., Suite 1400, Arlington Courthouse Plaza I, 2200 Clarendon Boulevard, Arlington, VA 22201 (US).</p>	<p>(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report.</p>	
<p>(54) Title: NON-BIOLOGICAL PATCH FOR HEMOSTASIS</p>  <p>(57) Abstract</p> <p>A hemostatic patch that is advantageously safe and inexpensive is created that comprises a sponge, and an effective amount of epsilon aminocaproic acid and a thrombin receptor activating peptide for promoting hemostasis. Epsilon aminocaproic acid is a hemostatic agent that inhibits fibrinolysis, accelerates the activity of thrombin and possesses antibacterial properties. Thrombin receptor activating peptide activates platelets and promotes platelet aggregation. The patch is particularly effective for decreasing bleeding of parenchymal organs, as well as for topical use particularly in a bandage form. The bandage form comprises a backing member (10) located contiguous with an exterior surface of the patch (20) and opposite the wound contacting surface of the patch (30). A flap (15) extends from the backing member (10) and a medically acceptable adhesive (25) can be applied onto the flap.</p>		

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NON-BIOLOGICAL PATCH FOR HEMOSTASIS

Field of the Invention

5 This invention relates generally to
hemostatic patches for arresting bleeding. More
specifically, the invention relates to patches or
matrices that include novel compositions of hemostatic
agents. Most specifically, the invention relates to
patches comprising a biodegradable matrix and
10 hemostatic agents epsilon aminocaproic acid and
thrombin receptor activating peptides sufficient to
arrest bleeding, and to methods of preparing and using
the same.

Background of the Invention

15 A hemorrhage of a blood vessel, body tissue,
organ or bone can result in blood loss leading to
hypovolemic shock and death. In hemophiliacs and
patients receiving anticoagulant medication, such as
often prescribed post-operatively for heart surgery,
the problem of rapid blood loss is even more acute.

20 A hemorrhage of a parenchymal organ, such as
the spleen, liver, lung or pancreas, which can result
from trauma or surgery, is particularly difficult to
treat. Parenchymal organs are difficult to ligate
because the tissue is easily torn, pulverized or

crumbled. As a result, surgeons often resort to the use of electrocautery, which can lead to further destruction of the patient's tissues.

Attempts have been made to devise a fast, effective and inexpensive method for curbing blood loss, including pastes containing coagulation-enhancing factors. One such coagulation enhancing substance employed to assist a cessation of bleeding or "hemostasis" is human fibrinogen, most commonly employed as a "fibrin glue".

Fibrin glue is composed of a mixture of human fibrinogen and bovine thrombin. It is sold as a kit containing separate vials of fibrinogen and thrombin solutions. These solutions are mixed together and applied to the wound in various ways, including as a paste, as a spray or on a patch.

Fibrin glue, however, is an inconsistent and ineffective therapy for hemostasis. The mixing, soaking, and coating of a patch with fibrin glue requires time-consuming and cumbersome procedures. Each of the preparation steps introduces potential errors and thus their efficacy varies with the experience of operating room personnel. Moreover, during the preparation of such solution, further hemorrhage occurs and the solutions are washed away by intense bleeding. Despite the headway made in fibrinogen compositions and surgical techniques, these pitfalls in achieving hemostasis underscore the need for development of a suitable product.

Another problem associated with fibrin glue and patches containing hemostatic agents is that they cause formation of abnormal adhesions. Adhesions can form when non-wounded, normal tissue contacts the hemostatic agents of the patch covering the wounded surface and then binds stably to it or "heals" to the wound abnormally. As an example of such a situation, an ulcerative, wounded intestine may abnormally adhere to a normal segment of intestine which overlies it

during its healing. The abnormal adhesion then causes the normal mobility of each of the intestinal segments to decrease, which in turn, impairs digestion.

5 An improvement over fibrin glue, marketed in Europe, consists of a biodegradable collagen patch onto which is impregnated bovine thrombin, aprotinin and human fibrinogen (the "TAF" patch). An example of a TAF patch is the TachoComb® patch marketed in Europe by Hafslund Nycomed Pharma, DE. The patch also contains
10 calcium chloride to enhance coagulation. In use, this patch is removed from its package, dipped into saline solution and applied to the bleeding organ with light pressure for at least five minutes. When the bleeding has stopped, the patch is left in place by the surgeon
15 and the cavity closed.

A major drawback both to fibrin glue and to the TAF patch is their reliance on essential active ingredients of human fibrinogen, a protein purified from human blood, and bovine thrombin, from cow blood.
20 Because of the high risk of HIV and hepatitis viral contamination, the Food and Drug Administration revoked the use of human fibrinogen in the United States in 1978. In addition to these safety concerns, human fibrinogen purified from human plasma is very
25 expensive.

Use of bovine or other species of thrombin similarly can introduce harmful viral contamination and possible transmission of bovine diseases, such as bovine spongiform encephalitis, particularly in Europe.

30 Thus, there is a continuing need to find alternatives to the use of animal origin proteins and other products in medical applications, including hemostatic products for use in humans.

Thrombin is a pivotal enzyme in platelet
35 activation and the formation of fibrin clots, but also has been more recently recognized for its role in mitogenesis and wound healing. High affinity thrombin receptors found on fibroblasts, and other cells,

including monocytes, neutrophils, keratinocytes and capillary endothelial cells have been implicated in initiating signals involved in platelet activation, mitogenesis, and wound healing. Vu et al. *Cell* 64: 1057 (1991); Carney et al., *J. Clin. Invest.* 89: 1469 at 1470 (1992).

Thrombin activates its high affinity thrombin receptor by proteolytically cleaving it to expose a new N-terminus. The cleaved terminus acts as a tethered ligand capable of self-activating at least the G-protein linked functions of the activated thrombin receptor. Vu et al., *Cell* 64: 1057 (1991). Recently, it was discovered that thrombin receptor can be activated by a peptide which mimics the new N-terminus created by the activation.

Thrombin receptor activating peptides (TRAPs) are a family of peptides of varying amino acid length which correspond to the new N-terminal region of the thrombin receptor. These synthetic or recombinant peptides mimic the activated form of the extracellular portion of the thrombin receptor protein and function as thrombin agonists.

U.S. Patent 5,256,766 describes pharmaceutical compounds containing TRAPs or "agonists" as useful to encourage blood clotting, for example, in localized application at internal bleeding sites of hemophiliacs. The agonists are disclosed as mimicking thrombin's ability to stimulate fibroblast proliferation and, concomitantly, platelet aggregation. TRAPs thus can be useful in promoting hemostasis and wound healing. The patent focuses upon systemic delivery of those compounds, but also discloses topical and local delivery among other routes of administration.

An effective hemostatic patch and hemostatic bandage is desired which is completely free of biological compounds such as thrombin and fibrinogen and the concomitant dangers of viral contamination.

Also, an effective means to provide TRAPS to a wound site in a mode which promotes hemostasis of the problematic hemorrhages of parenchymal organs and which adapts easily to body contours is desired. Thirdly, a need exists for a hemostatic patch or bandage that withstands elevated temperatures without requiring refrigeration and retains hemostatic efficacy.

Summary of the Invention

According to the present invention, an effective hemostatic patch is produced comprising a matrix and the hemostatic agents, epsilon aminocaproic acid and at least one thrombin receptor activating peptide. Importantly, the patch works effectively without resort to use of any exogenous human or animal protein, such as fibrinogen or thrombin. Thus the patch completely avoids introduction of unsafe contaminating viruses. The present hemostatic patch is thermally stable, antibacterial, inexpensive, easy to use, and can be provided in bandage form for topical use.

Brief Description of the Drawings

Figure 1 displays a control experiment showing thrombin activation at 37°C and physiological pH.

Figure 2 demonstrates the effects of pH on thrombin activation at 37°C.

Figure 3A and Figure 3B each show inhibition by EACA of *Staph. aureus* growth in the presence of various concentrations of EACA.

Figures 4A and Figure 4B each show inhibition by EACA of *E. coli* growth in the presence of various concentrations of EACA.

Figure 5A shows a side view of a bandage embodiment of the invention, including a patch of the

invention, while Figure 5B shows an elevation view of the bandage.

Detailed Description of the Invention

According to the present invention, a
5 hemostatic patch is provided that comprises a shaped structural element that is a biodegradable matrix, such as absorbable gelatin sponge, to which is applied the hemostatic agent, epsilon aminocaproic acid (EACA) and one or more thrombin receptor activating peptides
10 (TRAPs.) The term "TRAPs" as used herein refers to either an amount of a single type thrombin receptor activating peptide, or to an amount of two or more different thrombin receptor activating peptides used in combination.

15 A critical advantage of patch containing EACA and TRAPs is that it completely avoids proteins of human or animal origin. A fibrinogen or thrombin-containing hemostatic patch can be contaminated with harmful viruses, as discussed above. Further, in other
20 patches, the use of a non-human species of fibrinogen or thrombin triggers an immune response or allergic-like reaction in some persons.

Thus, a patch or bandage according to the invention can contain the non-biological agents EACA
25 and TRAPs dispersed within a matrix or applied to a surface of a matrix in amounts effective for both inhibiting fibrinolysis and stimulating clot formation.

Properties of EACA which contribute to the patch's effectiveness

30 EACA is an inhibitor of clot degradation. In the body, clot formation and clot breakdown are competing processes. EACA inhibits the production of plasmin, an enzyme that degrades clots. Plasmin degrades clots by solubilizing fibrin, an important
35 component of clots, in a process called fibrinolysis.

By inhibiting the formation of plasmin which breaks down clots, EACA inhibits fibrinolysis and drives the reaction conditions at the patch/biological interface in favor of clot formation. According to the present invention, the EACA in the patch functions as a hemostatic agent in a manner that approximates the effectiveness of fibrinogen, a coagulation factor used in other patches. A hemostatic patch according to the invention thus comprises an amount of EACA effective for inhibiting fibrinolysis.

It has now been discovered that, surprisingly, a patch containing EACA and TRAPs effectively stops bleeding of wounds, both externally and internally, due in part to a newly-identified property of EACA, namely, its ability to accelerate the activation of thrombin. Thus, it has been discovered that a patch comprising EACA exerts a dual hemostatic action by (1) slowing clot degradation by inhibiting plasmin formation and (2) accelerating clot formation by activating thrombin. It is believed that the presence of effective amounts of EACA increases the alkalinity in the matrix and the local environment of the patch as it solubilizes upon its contact with blood and as the blood penetrates the patch. This increased alkalinity enhances activation of the thrombin present in the blood and promotes clotting.

Supporting this hypothesis are data showing the pH dependence of thrombin activation. In comparison with thrombin activation measured in the absence of EACA (Figure 1, closed boxes), EACA greatly increases thrombin's activity (Figure 2). This phenomenon holds true whether the EACA acts on thrombin present in the blood endogenously or on thrombin that is supplied externally in a patch.

Yet another surprising advantage of EACA contributes to the effectiveness of a hemostatic patch according to the invention. EACA possesses antibacterial properties. According to the present

invention, it has been demonstrated that EACA exerts dose-dependent inhibition of both *S. aureus* and *E. coli* growth (Figures 3A, 3B and 4A, 4B, respectively). Therefore, the EACA/matrix patch according to the present invention is very desirable for its antibacterial effects on microorganisms present at the wound site where a patch is applied. The combined effects of EACA's and TRAPs' advantageous properties provide a non-biological patch which functions very well to promote hemostasis safely and quickly.

Properties of TRAPs which contribute to the patch's effectiveness

TRAPs, as discussed above, are known to stimulate fibroblast proliferation, activate platelets and promote wound healing. The signaling events initiated by TRAPs, particularly triggering of platelet activation, encourage blood clotting and thus are useful in promoting rapid hemostasis. Platelet activation promotes platelet aggregation, which is an integral step in the process of hemostasis. TRAPs provide many of the same hemostatic biological activities of thrombin, but without the hazards of contamination associated with a biological agent.

TRAPs further possess the advantage of being synthesized easily and inexpensively. The peptides for use in the patch of the invention are prepared using standard solid phase (or solution phase) peptide synthesis methods known in the art.

In addition, the DNA encoding these peptides may be synthesized inexpensively using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. Recombinant production is disclosed in U.S. Patent 5,256,766, that specific production disclosure of which is expressly incorporated by reference herein.

U.S. Patent 5,256,766 to Coughlin discloses representative TRAPs which may be incorporated into a patch according to the invention. That patent is expressly incorporated herein by reference in its entirety. TRAPs which may be useful in the present patch include peptides capable of activating thrombin receptor, such as the agonists identified in the Coughlin patent by the formula $AA_x--AA_y--(AA_i)_n--z$.

Other TRAPs which have been disclosed which activate fibroblasts and are implicated in wound healing include peptides TRAP 508-530, amino acids AGYKPDEGKRGDACEGDSGGPFV; and TRAP 517-530, amino acids RGDACEGDSGGPFV. Carney et al. *J. Clin Invest.* 89:1469-1477 (1992); and Cromack et al., *J. Surg. Res.* 53: 117 (1992), both of which publications are expressly incorporated by reference in their entirety.

Accordingly, suitable TRAPs useful in the present invention, for example, include peptides SFLLRNPNDKYEPF; SFLLRNPNDKYEP; SFLLRNPNDKYE; SFLLRNPNDKY; SFLLRNPNDK; SFLLRNPND; SFLLRNP; SFLLRNP (TRAP 1-7); SFLLRN (TRAP 1-6); SFLLR; SFLL; and SFL, and the amidated forms thereof. Other TRAPs that advantageously can be used in the present invention include the peptides TRAP 508-530, amino acids AGYKPDEGKRGDACEGDSGGPFV; and TRAP 517-530, amino acids RGDACEGDSGGPFV, referenced above.

Because TRAPs are small peptides, they are more stable than large proteinaceous platelet activating agents, such as thrombin. The stability of TRAPs contributes to the properties of the present patch which permit it to be stored without refrigeration, as discussed below.

Further Advantages of the Invention

In one embodiment of the invention, a patch is made that is particularly suitable for internal/surgical use which avoids adhesions. In such a patch, the EACA and TRAPs components are applied to a

single side of a flat patch, namely, the wound-contacting surface. Other optional additive(s) can be added to the wound-contacting surface, as well. By virtue of its uni-sided construction and the nature of its active agents, the present patch is much less susceptible to forming undesirable adhesions between adjacent tissue surfaces. Another advantage of the patch results from the stability of the patch's key active agents as described previously. Thus, the patch of the present invention need not be stored at low temperatures, e.g., in a refrigerator. This feature permits use of the patch in field situations, such as for military or emergency use.

Another important advantage of the present invention is flexibility, that is, a patch is provided that easily conforms to the contours of an organ or biological surface, making the manipulation of applying the patch quicker to perform. As a result, there is less overall blood loss to the patient and less time is spent in surgery.

A patch containing TRAPS would also be useful in patients taking dicumarol which inhibits the liver's ability to synthesize prothrombin.

Matrix Composition

As employed in any of the present embodiments of the invention, a biodegradable "matrix" is a porous support, selected from, but not limited to, the group consisting of absorbable gelatin sponge, calcium alginate, calcium/sodium alginate, collagen, and oxidized regenerated cellulose. A matrix of other forms of collagen, such as cross-linked collagen, esterified collagen or chemically modified collagen as taught by U.S. Patent No. 4,390,519 to Sawyer, and other conventional matrices utilized in hemostatic patches, also can be used according to the present invention. Four examples of matrices that are particularly advantageous for use with EACA and TRAPS

include absorbable gelatin sponge, calcium alginate, calcium/sodium alginate, and collagen. A number of such matrices are commercially available.

Shape

5 According to the present invention, a hemostatic matrix containing non-biological ingredients is provided in a flat patch form, particularly suitable for internal use, or in bandage form for topical use. In a preferred embodiment, the EACA and TRAPs are
10 provided on a flat flexible matrix which is biodegradable and absorbable. The matrix advantageously has an uncompressed thickness of about 4-10 mm, or a compressed thickness of about 2-10 mm, advantageously 2-5 mm, a length of about 1 to 20 cm,
15 preferably 4-10 cm and a width of about 2-10 cm, preferably 3-7 cm. The matrix, of course, may be cut to a desired length and constructed into a patch or bandage, as desired.

Size

20 A patch in size and shape according to the intended use. Moreover, a standard size rectangular patch, 10 x 5 cm, having an uncompressed thickness of about 4-10 mm, or a compressed thickness of about 2-10 mm, advantageously 2-5 mm, may be cut to size with a
25 pair of scissors. A preferred matrix to which EACA, TRAPs and other additives according to the invention are applied includes gelatin foam, preferably provided in a compressed form. More preferably, a GelFoam®
30 matrix that is compressed to at least one-half its original thickness may be used.

 Also, a patch may be spherically, conically, cuboidally tubular or cylindrically-shaped or prefabricated into small squares, such as for packing into a body cavity. Such an embodiment is useful for
35 example, for a dental cavity resulting from tooth extraction. Additionally, the patch can be configured

into a tampon, for example, for epistaxis (profusely bleeding nostril) or other void.

One or more additional layers of wound dressing material, preferably a layer which aids in absorption of blood or other exudants, can be applied to a patch. Such an additional layer can be made as an integral part of the patch, thereby creating a thicker patch. Alternatively, the layer may be applied as a supplement to the back side (non-wound contacting surface) of a patch according to the invention. Particularly for topical use, the layer(s) can contain superabsorbents to wick exudant solution from the wound site. It is advised that for patches intended for internal-surgical applications, where an added layer(s) is integral with the patch, the layer(s) should be both biodegradable and pharmaceutically acceptable.

The patch can be designed to facilitate its application to anastomose or fuse ends of a blood vessel or other body lumen which has been severed surgically or otherwise. To apply a patch for anastomosis, a rectangular GETR patch, for example, is wrapped around the external surface of the ends of a Dacron® graft. A patch of tubular configuration may also be used. When the graft is positioned into place, the patch accelerates fibrin growth into the graft to seal the graft in place (hemostatically and hermetically).

A kit is provided that contains a graft and a patch according to the present invention that is designed for fitting with the ends of the graft. Alternatively, a kit is provided having a patch of the present invention pre-fitted onto at least one end of a graft.

Preferably, a wound-contacting surface of the patch is coated with a color indicator to assist the user, such as yellow vitamin B₂ (riboflavin) or a suitable dye, for example, hemin. By color coding the patch, the user knowingly avoids touching or otherwise

contaminating the wound-contacting surface of the patch.

Bandage Form

A patch embodiment intended for topical applications additionally can be applied with an adhesive tape, as a bandage form, where the patch is adhered to an adhesive backing. Preferably the adhesive used to secure the patch is porous in areas which contact the skin. An embodiment of the present invention provides a thin film wound dressing or bandage for application to skin surfaces to provide a sterile mechanical barrier to all types of infectious agents.

Preferably, the "bandage" embodiment, shown in Figure 5, comprises a backing member 10 located contiguous with an exterior surface of the patch 20, which is opposite that of the wound contacting surface 30. In a preferred embodiment, the backing serves to prevent passage of the EACA, TRAPS, optional additive(s) and other biological exudants through the exterior surface 30, as well as providing a barrier to entry of infectious microorganisms into the patch and underlying wound. The backing also provides physical support and protection for the patch and underlying wound, and preferably is fixedly secured to the exterior side of the patch, for example, with a medically acceptable adhesive. Various occlusive and non-occlusive, flexible or non-flexible backing members can be used in the adhesive bandage of the invention. Preferable for use in this context are several polyurethane films that have been specifically adapted for wound dressings and other medical uses. These films are typically used in thicknesses of less than 2 mm and allow the free diffusion of oxygen, water vapor and other gasses through their molecular matrices. In addition, these films are impermeable to both liquids and all known microbial disease vectors.

Other suitable backings can include polyethylene, acrylic, silicone, cellophane, cellulose acetate, ethylcellulose, plasticized vinylacetate-vinylchloride copolymers, polyethylene terephthalate, nylon, polyethylene, polypropylene, polyvinylidenechloride, paper, cloth, aluminum foil and other conventional backing materials. Preferably, a flexible backing material is employed to permit the bandage to readily conform to the contour of the patient's body member to which the patch is applied.

In providing a bandage according to the invention, a flap 15 extends from the backing member, beyond the region of the patch. In another embodiment, the flap(s) is fixedly secured to patch itself, preferably on the sides of the patch.

Advantageously, at least two flaps are provided which extend from the backing member, on opposite sides of the length, or of the width of the patch member. Alternatively, a plurality of contiguous flaps may extend in overhanging fashion outwardly from the backing member beyond the patch, such as shown in Figure 5.

Where a flap extends outwardly from a single side of the backing member, the flap preferably is sufficiently long to permit its encircling an appendage, such as a finger, limb, or torso, and adhering to the backing member on the opposite side of the patch.

A medically acceptable adhesive 25 is applied onto the flap(s), to permit adherence between the backing member and the patient's skin. Preferably, the flap(s) and the backing member are made of material which permits the skin to breathe. The backing and flap material can be made of the same or different materials.

A removable "pull-tab" 40, comprising a layer of cellophane, plastic or the like, is applied to the flap(s) to prevent it (them) from sticking to other

surfaces prior to use. To use the topical adhesive bandage of the invention, the pull-tab layer(s) are readily peeled off just before application of the bandage to the body. Then the bandage is applied with the wound-contacting surface facing downward in direct contact with the wound. The flap(s) preferably does not contact the wound, but instead is applied to a region(s) of normal tissue adjacent the wound to secure the patch member in place.

Amount of Components

EACA is applied to the wound contacting surface of the matrix in an amount effective, preferably about 10-100 mg/cm², more preferably 60-70 mg/cm². TRAPs are applied to the wound matrix in amounts effective, preferably about 0.25-100 mcg/cm². More preferably, TRAP 508-530 or TRAP 517-530 is applied in amounts ranging between 0.25-25 mcg/cm², or 1.0-10 mcg/cm², while TRAP 1-7 or TRAP 1-6 is applied in amounts ranging between 1-100 mcg/cm², or 10-50 mcg/cm².

Additives

Optionally, one or more additives, including a calcium ion source, RGD peptide, RGDS peptide, ADP, protamine sulfate and buffer, also are applied to the wound-contacting surface of the patch or dispersed in the patch matrix in amounts described in the embodiments below.

Additional hemostatic agents can be applied to the patch in amounts effective for stimulating hemostasis, including, but not limited to: calcium, sodium, magnesium or other ions that stimulate hemostasis. In terms of ion additives, calcium chloride is generally a preferred additive for introducing calcium ion into the patch.

"EACA analogs," or compounds that possess a similar hemostatic activity and a chemical structure to that of EACA, can be used instead of, or in addition to, EACA in a patch according to the invention. Possible EACA analogs contemplated for addition to a matrix, in amounts effective to stimulate hemostasis include EACA derivatives having bioisosteric functional groups. EACA's carboxylic acid group can be substituted, for example, by sulfonic or sulfinic acid ($-SO_2H$ and $-SO_3H$) or phosphonic acid groups. Examples of analogs include, but are not limited, to 5-aminopentanoic acid, 7-aminoheptanoic acid, 8-aminooctanoic acid, provided that these compounds exert a hemostatic activity.

A "sterile buffer" which is pharmaceutically acceptable and capable of buffering the local pH in the patch to alkaline conditions, (i.e., between a pH of 7.0-9.0, more advantageously pH 7.00-8.20, and even further advantageously, 7.6-8.2), is suitable as an additive to enhance the activity of exogenous thrombin. For example, Tris buffer is an effective thrombin-enhancing sterile buffer, as shown in Figure 2, open diamond-shaped graphical plot. Other sterile buffers that buffer the pH in this range are contemplated, such as Hepes buffer, for example. Accordingly, Tris or other buffer both can be provided in the matrix of the patch.

By way of example, a preferable embodiment of the invention provides a patch comprising a matrix of absorbable gelatin sponge "G," a hemostatic agent, EACA "E" and a thrombin receptor activating peptide "t."

This embodiment, "GEt", preferably also can contain calcium, "GEt(Ca++)". Advantageously, the GEt or GEt(Ca++) patch provides rapid, effective hemostasis, has good thermal stability and can be stored for months to a few years without refrigeration and without losing its effectiveness. The GEt and GEt(Ca++) patches are useful for field and emergency use, since each may be stored in a ready-to-use state for a lengthy period, without refrigeration. Both also are much less expensive to make than patches which contain fibrinogen.

The many representative embodiments of the present invention are referred to herein most easily by acronyms, e.g., GEt. These acronyms are indicative of the individual components (Table 1) found in the patches created in accordance with the invention.

Table 1.

PATCH COMPONENT CODES:

G	= gelatin foam patch alone, e.g., Gelfoam®
CA	= calcium alginate
CVA	= calcium/sodium alginate, e.g., Kaltostat®
C or CVC	= collagen or collagen(Helistat®),
respectively E	= EACA
t	= thrombin receptor activating peptide or
peptides	
(Ca++)	= calcium
R	= RGD peptide
P	= protamine sulfate
B	= buffer

In the GEt and GEt(Ca++) patches, and all patches described herein that employ an absorbable gelatin sponge^{USP} as a matrix, the matrix is advantageously a flat layer of gelatin foam, more advantageously, Gelfoam®, and even more advantageously, compressed gelatin foam or compressed GelFoam® (UpJohn Co.) *Physician's Desk Reference* 2451, 47th Edition Dowd (ed.), Medical Economics Data (1993). The

effectiveness of patches of the present invention in promoting clot formation is enhanced by the lattice structure of the gelatin foam, which promotes enzyme substrate interactions. In particular, the gelatin foam structure enhances contact between EACA and TRAPs in the patch with endogenous fibrinogen and thrombin present in the blood exuding from the wound.

In yet another embodiment, "CVAEt," a patch is provided having a matrix composed of calcium-sodium alginate "CVA" or calcium alginate "CA," with applied agents EACA and TRAPs. It is understood that calcium alginate can be substituted for calcium/sodium alginate in the discussion and examples hereafter, without substantial differences in results. The embodiment "CVAEt", already contains calcium ions. Similar to the GEt patch, the CVAEt patch can include additional hemostatic agents and/or additives as described herein.

In another embodiment, an effective amount of the active peptide, RGD, "R" or RGDS effective to stimulate wound healing is added to a patch comprising GEt or CVAEt, and thus such a patch is designated as GEtR or CVAEtR. The tripeptide RGD is composed of arginine, glycine and aspartic acid, and optionally serine "RGDS," and is the active site of fibrinogen and fibronectin. RGD accelerates wound healing and is believed to stimulate fibroblast migration.

The RGD additive is also much less expensive relative to that of fibrinogen. RGD can be synthesized easily using conventional solid phase chemistry at a fraction of the cost of obtaining fibrinogen, which currently must be obtained by purification from a natural source.

In still another embodiment, an amount of the agent adenosine dinucleotide phosphate (ADP) effective to stimulate platelet aggregation is added to any of the aforementioned patches comprising EACA, TRAPs and a matrix. Tedd, Cook et al., *Thromb. Haemost.* 70:531-539 (1993) discloses that 0.2-0.5 mM ADP and 1-10 mM

TRAP exert a platelet aggregating effect within 2 minutes. Accordingly, the range of ADP may be 5 times less than that of the overall range of TRAPs (which may be, as indicated, 0.25 at the lowest dosage -
5 100mcg/cm² at the highest dosage), and thus range of ADP is between 0.05 - 20 mcg/cm².

In yet another embodiment, an amount of the agent protamine sulfate "P" effective to neutralize heparin present in the local environment of the patch
10 is added to any of the aforementioned patches comprising EACA and a matrix. Protamine sulfate neutralizes heparin or vitamin K antagonists that are present in the blood of certain patients or animals being treated with a hemostatic patch.

15 A patch comprising GEtP or CVAEtP, for example, is prescribed for persons undergoing parenteral therapy with heparin. A patch containing protamine sulfate is preferably stored at refrigerated temperatures of 2-8 degrees Celsius to maintain the
20 activity of protamine sulfate.

An additional advantage of the patches according to the present invention is that the matrices, such as absorbable gelatin sponge or calcium alginate, and especially EACA, TRAPs and the additive,
25 RGD, all are relatively inexpensive to make. It is estimated that production of a "standard-size" rectangular patch of about 10 x 5 cm, having a thickness of about 2.5 mm would cost substantially less than a TAF patch of the same size.

30 Patches according to the present invention are efficacious in inducing hemostasis in freely bleeding lesions of the spleen, liver and kidney of an anesthetized pig. Surgical lesions induced in parenchymal organs of pigs provide a good model system
35 for hemostasis in the analogous human organs as evidenced by preclinical studies performed on pigs and dogs for the TachoComb patch. Schiele et al., *Clinical Materials* 9:169 at page 172 (1992). See also,

SWINE AS MODELS IN BIOMEDICAL RESEARCH, Swindle, M.,
Iowa State Univ. Press (1992).

Applying the Patch

5 A hemostatic patch according to the present
invention is applied to a wound by placing the wound-
contacting surface on the wound. Then, the patch is
maintained in contact with the wound for a period of
time sufficient for clotting to occur at the interface
between the hemostatic patch and the wound, and
10 sufficient for bleeding to be substantially arrested.
Preferably the patch is maintained in contact with the
wound surface for a period of about 3-20 minutes,
advantageously 3-10 minutes, and more advantageously,
3-5 minutes. Where EACA, TRAPs and calcium chloride
15 all are present on the matrix, the time period is
preferably about 5 minutes. The patch is held in
place against the biological surface preferably with
light pressure, preferably by means of a sterile
saline-soaked sponge. Alternatively, the patch may be
20 held in place simply by applying pressure to the patch
by means of a gauze or other dry sterile material.
Depending on the location of the wound, a bandage,
including an elasticized bandage, that could be
manufactured as an integral part of the patch, can be
25 wrapped around the patch so as to provide light
pressure on the wound site.

In addition to inducing hemostasis, a patch
according to the present invention is useful for
hermetically sealing body tissue. For example, when
30 air leaks from a wound in the lungs, a patch is applied
to the surface surrounding the wound, held in place
with light pressure for a period of time adequate to
induce hemostasis, as discussed above. During that
time, in addition to hemostasis, a hermetic seal forms.

35 Prior to applying the patch, it may be
preferable to soak the patch in sterile saline
solution. Such a step is not required, however. Use

of a hemostatic patch according to the invention, without first soaking in saline solution permits quick and simple application of the patch in field situations, such as may be encountered by an emergency medical technician or a military healthcare worker.

In one embodiment, the patch is contained within a sealed sterile package which facilitates removal of the patch without contamination. Such a package for example, can be an aluminum foil pouch or other conventional material that is easily sterilized. Radiation, advantageously gamma radiation, is applied to sterilize the patch and packaging material together.

In another embodiment, a container having dual compartments is provided. A first compartment contains distilled water, sterile saline or a sterile buffer, while the second compartment contains a patch according to the invention. In field use, the patch of the second compartment can be readily dipped into an opened first compartment and subsequently applied to the wound.

A preferred use of a patch according to the present invention is to inhibit or completely stop bleeding of a parenchymal organ, such as the liver, kidney, spleen, pancreas or lungs. Additional uses for such a patch include curbing bleeding of tissues during types of surgery such as, but not limited to, internal/abdominal, vascular (particularly for anastomosis), urological, gynecological (particularly for an episiotomy), thyroidal, neurological, ear nose and throat, tissue transplant, and dental surgeries.

Another use of a hemostatic patch includes topical treatment, such as for burn or tissue transplants. A patch intended for topical use according to the invention preferably contains additional additives, such as anti-infection medicaments. Bactericides, fungicides and wound healing agents can be added, as well. Neomycin and bacitracin are examples of certain additives that are

incorporated into a patch intended for topical use, in addition to the antibacterial properties contributed by EACA, as discussed above.

5 A hemostatic patch of the invention also is useful for treating animals, preferably humans or other mammals. For example, companion, livestock and wild animals can be treated with a hemostatic patch.

How to Make the Patch

10 EACA, TRAPS and other hemostatic agents or additives described as components of a patch according to the invention can be applied to the matrix by any of several methods, all of which would be performed most advantageously under sterile conditions. It is
15 understood that conventional methods of applying the hemostatic agents and additives to a matrix comprising EACA besides those described herein can be performed as well.

Advantageously, EACA and TRAPS are applied to one side of a flat matrix, which then is designated as
20 the wound-contacting surface. This can be accomplished by spraying EACA in powder form, or TRAPS in lyophilized form, onto the patch. Or, TRAPS can be dissolved in double distilled water or in a solution of calcium chloride, and sprayed onto a wound-contacting
25 surface of the patch, such that there will be separate layers of TRAPS and EACA. As another alternative, a solution of EACA and TRAPS can be coated onto a matrix and dried by lyophilization or by conventional means.

30 In another method of applying EACA, a matrix is dipped completely or partially into a sterile solution of EACA and TRAPS such that a sufficient amount of EACA and TRAPS accumulates within the matrix effective to provide hemostasis as according to the invention. In making a patch intended for internal
35 use, the matrix is preferably not dipped completely or otherwise saturated with EACA and TRAPS; rather, the

compounds are applied to a single side by any of the alternate ways described above.

Coatings to facilitate adherence could be utilized, as well. In a more advantageous embodiment, the matrix is coated with a coating layer prior to application of EACA and TRAPs. In a further advantageous embodiment, the matrix is treated before and after addition of EACA and TRAPs with a coating layer, preferably made of a compound in solution with an ion additive, such as calcium (i.e., calcium chloride solution).

The drying step is accomplished by lyophilization, preferably. Other drying procedures appropriate for a material containing peptides can also be employed, so long as the drying treatment does not denature the peptides or render them inactive when exposed to animal blood. Alternatively, the patch is conventionally dried, by maintaining it at room temperature for a period of 1-3 hours, followed by refrigeration overnight.

In yet another embodiment, an agent added to a matrix, containing EACA, TRAPs, calcium chloride and optional additives, includes an amount of protamine sulfate effective to neutralize heparin in the local environment of the patch. Protamine sulfate is added in an amount between 1-15 mg/cm² of said matrix, preferably in an amount between 2-5 mg/cm² of a wound contacting surface of the matrix.

Likewise, the peptide arginine-glycine-aspartic acid (RGD) or arginine-glycine-aspartic acid-serine (RGDS) can be dissolved in double distilled water and sprayed onto a wound-contacting surface of the patch. A patch advantageously contains an amount of RGD effective to enhance clot formation. RGD or RGDS is applied to a patch advantageously in an amount between 110-130 mg/cm². Thus, a standard size patch would contain about 1-10 mg/patch or about 5-7 mg/patch of RGD or RGDS.

It should be noted that, like EACA, other hemostatic agents or additives described in the foregoing paragraphs can be applied to a matrix as a layer, for example, by spraying them onto the wound-contacting surface of the matrix in dried forms. Alternatively, a matrix can be dipped or coated with a solution containing the hemostatic agent/additive.

It is desirable that the matrix and agents commingle, particularly when the patch is exposed to a body fluid such as blood, which permits the dried agents to solubilize and mix. Thus, a patch can be provided wherein the hemostatic agent or mixture of hemostatic agents are absorbed into the pores or interstices of the matrix. Or, the agents are layered on a single surface of the matrix and upon addition of body fluid, the desired commingling is achieved.

The matrix can be coated with appropriate hemostatic agents described in the above embodiments on one or all surfaces. For example, in an embodiment intended for packing a void in body tissue, the patch is coated or saturated with hemostatic agent(s)/additive(s) on all surfaces.

In a preferred embodiment, the hemostatic agents and additives are coated on only one surface, the wound-contacting surface. Alternatively, a patch which is saturated with agents and additives is provided with a barrier layer positioned so as to prohibit the diffusion of agents and additives to an opposite surface of the patch from that of the wound contacting surface. Such a barrier may be made of hyaluronic acid or similar material. These embodiments advantageously avoid adhesion formation resulting from hemostasis occurring between the wound and a non-wounded tissue in the vicinity of the patch.

A kit according to the invention comprises any of the above described hemostatic patch embodiments (which vary in ways including hemostatic agent(s) and additive(s) utilized, shape or size) according to the

invention and a package, wherein the patch is contained within a sealed sterile package which facilitates removal of the patch without contamination. The kit can contain multiple patches, preferably wherein each patch is contained within a separate sealed sterile package or compartment. A kit designed for field/military use can, in addition to a hemostatic patch, further include disposable pre-sterilized surgical instruments, such as a scalpel, clamp, tourniquet, elastic or inelastic bandage, or the like.

Another type of kit comprises a patch containing agents added to the matrix including EACA, TRAPs, calcium chloride, and protamine sulfate. Such a kit can be prescribed, for example, to patients requiring anticoagulant therapy, to avert the risk of serious bleeding which can occur from a minor injury. Protamine is particularly advantageous to facilitate patients who are anticoagulated for myocardial, pulmonary, or vascular disease or other medical complications in which prevention or prolongation of coagulation is of advantage. The availability of such a patch can reduce postoperative hospitalization for patients on dicumarol who underwent surgery.

The present invention is further described with reference to the following, illustrative examples. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art of the invention. Although any methods and materials similar or equivalent to those described herein can be used in the practice of the invention, the preferred methods and materials have been described. Unless mentioned otherwise, the techniques employed or contemplated herein are standard methodologies well known to one of ordinary skill in the art. The materials, methods and examples are illustrative only and not limiting.

EXAMPLE 1: THE EFFECTS OF EACA ON THROMBIN ACTIVATION

A two-part experiment was designed to test whether thrombin activation in the presence of EACA (A) is accelerated and (B) is pH dependent.

A. Effect of Time Incubated at 37° C.

The first part of this study examined activation of thrombin and its degradation in H₂O after incubation at 37°C. The assay used was a colorimetric cleaving of a tripeptide, TFA-phe-pro-arg-AFC, where the AFC is the colorimetric tag. Seventeen mg of this substrate was dissolved in 200μl DMSO. Thrombin was made up as 10 units/ml. The "TEST" solution contained 100μl substrate and 200μl of the thrombin solution; a blank contained the same amount of substrate and 200μl of H₂O.

Figure 1 labeled as "ACTIVATION OF THROMBIN SOLUTION AT 37°C" shows the results of that experiment. The optical density in all of these experiments is an indication of the color and therefore the amount of cleavage of the enzyme that has taken place.

The slope of the black-box line indicates that thrombin activation of thrombin dissolved in H₂O takes place slowly over a 172 minute time period. The blank, containing substrate and H₂O, shows no change in optical density, indicating that no activation, or cleaving of the peptide has occurred.

B. Thrombin Activation by EACA: A pH Effect

In this experiment, the hypothesis that the activation of thrombin by EACA was due to EACA's effect of increasing pH was tested.

All solutions were prepared at the same concentration as indicated in part A above, except EACA which was made up at a concentration of 50 mg/ml. The following samples were prepared:

1. 50μl Thrombin + 925μl H₂O + 25μl substrate
2. 50μl Thrombin + 925μl Tris buffer @ pH 7.02 + 25μl substrate

3. 50 μ l Thrombin + 925 μ l Tris buffer @ pH 7.62 + 25 μ l substrate
4. 50 μ l Thrombin + 925 μ l Tris buffer @ pH 7.80 + 25 μ l substrate
5. 50 μ l Thrombin + 925 μ l Tris buffer @ pH 8.01 + 25 μ l substrate
6. 50 μ l Thrombin + 425 μ l EACA sol. + 500 μ l H₂O + 25 μ l substrate
7. 50 μ l Thrombin + 425 μ l EACA sol. + 500 μ l Tris buffer @ pH 7.02 + 25 μ l substrate
8. 50 μ l Thrombin + 425 μ l EACA sol. + 500 μ l Tris buffer @ pH 7.62 + 25 μ l substrate
9. 50 μ l Thrombin + 425 μ l EACA sol. + 500 μ l Tris buffer @ Ph 7.80 + 25 μ l substrate
10. 50 μ l Thrombin + 425 μ l EACA sol. + 500 μ l Tris buffer @ Ph 8.01 + 25 μ l substrate

Each tube was placed in a 37°C. water bath and removed periodically to be read each 5' for a total of 60'. Results are summarized in Figure 2. In the legend, samples 1-10 listed vertically in the legend correspond to samples 1-10 immediately above, while "T" represents thrombin.

The results indicate clearly that the action of EACA is a pH effect and that Tris buffer-adjusted solutions had a similar effect as the pH was increased. In all cases, the plateau may not be accurate since the saturation of the instrument occurs near to the maximum optical density recorded.

At 37°C, the results indicated clearly that the action of EACA is a pH effect. Calcium ion appears to enhance this pH-mediated activation.

EXAMPLE 2: EACA EXERTS AN ANTIBACTERIAL EFFECT

EACA was shown to inhibit both *Staph. aureus* and *E. coli* in a dose-dependent manner by the following method.

Culture plates and EACA discs were prepared as follows: Whatman filter paper discs of 5.4 cm in

diameter and 22.9 cm² in area were placed in beakers of almost the same diameter. EACA (229 mg) was dissolved in 250μl of double distilled H₂O and used to make the final concentrations. All concentrations of EACA were applied in 250μl of H₂O. Concentrations of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg/cm² were prepared. After application of EACA solutions, the discs were allowed to dry and frozen to ensure stability.

Discs for application to agar plates were made with a paper punch, at a size of about 6.35 mm. Agar plates were poured in two increments.

A first increment of 1.5% Brain-Heart Infusion agar was prepared and autoclaved. After cooling to approximately 55°C., 12 ml were added to each 100 mm x 15 mm petri dish. Plates were allowed to cool to room temperature, wrapped in parafilm and refrigerated. Brain-Heart Infusion broth was prepared and autoclaved. When the temperature was cooled to room temperature, a 1 ml aliquot of *Staph. aureus* or *E. coli* was added and the broth incubated overnight at 37°C.

The following day, a second increment of 1.2% Brain-Heart Infusion agar was prepared and when cooled to 48°C after autoclaving, 2 ml of each culture was added to separate flasks of agar and 1 ml of these mixtures was added to each culture plate. This top layer was allowed to harden at room temperature. Two sets of five discs containing EACA at varying concentrations were added to each plate, in addition to a control disc containing zero mg/cm² EACA. The complete results are listed in Table 2. Figure 3A and Figure 3B each show inhibition by EACA of *Staph. aureus* growth graphically, for each set of various concentrations of EACA, while Figures 4A and Figure 4B each show inhibition by EACA of *E. coli* growth for each set of varying concentrations of EACA.

Results of observation and measurement of the zone inhibition reveal that in almost all instances, there is an incremental change in this zone of inhibition related to the concentration of EACA. The exceptions are that the 60 mg/cm² did not follow the trend, but was equal to or decreased in relation to the 40 mg/cm². The 90 and 100 mg/cm² zones were not always increases. The consistency of these variations appear to be related to the disc preparation rather than a biological variation.

5

10

TABLE 2.

Results: Inhibition of Staph. aureus (Plates 1-6)
and E. coli (Plates 7-12) Growth by EACA

Date	Plate Number	Organism	Conc. of EACA in mg/cm ²	DIAMETER OF INHIBITION	% > CONTROL	% OF MAXIMUM
10/22/93	1	<i>E. coli</i>	control	6.35	0.00	77.00
			10	6.95	9.40	84.20
			30	7.65	20.50	92.70
			50	7.75	22.00	93.90
			70	8.25	29.90	100.00
			90	7.50	18.10	90.90
10/22/93	2	<i>E. coli</i>	control	6.35	0.00	70.20
			10	6.70	5.50	74.00
			30	8.55	34.60	94.50
			50	8.60	35.40	95.00
			70	9.05	42.50	100.00
			90	8.35	31.50	92.30
10/22/93	3	<i>E. coli</i>	control	6.35	0.00	70.60
			10	6.70	5.50	74.40
			30	7.05	11.00	78.30
			50	7.10	11.80	78.80
			70	9.00	41.70	100.00
			90	8.25	29.90	91.70
10/22/93	4	<i>E. coli</i>	control	6.35	0.00	77.40
			20	7.05	11.00	86.00
			40	7.70	21.30	93.90
			60	7.70	21.30	93.90
			80	8.20	29.10	100.00
			100	7.75	22.00	94.50
10/22/93	5	<i>E. coli</i>	control	6.35	0.00	78.40
			20	7.70	21.30	95.10
			40	7.75	22.00	95.70
			60	7.45	17.30	92.00
			80	8.10	27.60	100.00

Date	Plate Number	Organism	Conc. of EACA in mg/cm ²	DIAMETER OF INHIBITION	% > CONTROL	% OF MAXIMUM
			100	8.10	27.60	100.00
10/22/93	6	<i>E. coli</i>	control	6.35	0.00	76.50
			20	7.60	19.70	91.60
			40	8.10	27.60	97.60
			60	7.90	24.40	95.20
			80	8.25	29.90	99.40
			100	8.30	30.70	100.00
10/22/93	7	<i>S. aureus</i>	control	6.60	0.00	77.20
			10	7.55	14.40	88.30
			30	8.20	24.20	95.90
			50	7.65	15.90	89.50
			70	8.55	29.50	100.00
			90	7.90	19.70	92.40
10/22/93	8	<i>S. aureus</i>	control	6.55	0.00	80.90
			10	7.10	8.40	87.70
			30	7.00	6.90	86.40
			50	7.15	9.20	88.30
			70	7.75	18.30	95.70
			90	8.10	23.70	100.00
10/22/93	9	<i>S. aureus</i>	control	6.55	0.00	81.90
			10	7.00	6.90	87.50
			30	7.20	9.90	90.00
			50	7.45	13.70	93.10
			70	7.85	19.80	98.10
			90	8.00	22.10	100.00
10/22/93	10	<i>S. aureus</i>	control	6.60	0.00	79.00
			20	8.05	21.90	96.40
			40	8.30	25.80	99.40
			60	8.10	22.70	97.00
			80	7.55	14.40	90.40
			100	8.35	26.50	100.00
10/22/93	11	<i>S. aureus</i>	control	6.50	0.00	83.90

Date	Plate Number	Organism	Conc. of EACA in mg/cm ²	DIAMETER OF INHIBITION	% > CONTROL	% OF MAXIMUM
			20	7.20	10.80	92.90
			40	7.70	18.50	99.40
			60	7.30	12.30	94.20
			80	7.40	13.80	95.50
			100	7.75	19.20	100.00
10/22/93	12	<i>S. aureus</i>	control	6.50	0.00	79.30
			20	6.75	3.80	82.30
			40	8.05	23.80	98.20
			60	8.00	23.10	97.60
			80	8.20	26.20	100.00
			100	7.20	10.80	87.80

CLAIMS

We Claim:

1. A hemostatic patch comprising a biodegradable matrix to which matrix is applied hemostatic agents
5 comprising an amount of epsilon aminocaproic acid effective for inhibiting fibrinolysis and an amount of a thrombin receptor activating peptide effective for promoting platelet aggregation.
2. A hemostatic patch comprising a biodegradable
10 matrix having a wound-contacting surface, to which surface is applied hemostatic agents comprising an amount of epsilon aminocaproic acid effective for inhibiting fibrinolysis and an amount of a thrombin receptor activating peptide effective for promoting
15 platelet aggregation.
3. A hemostatic patch according to claim 2, wherein the epsilon aminocaproic acid is applied in an amount between 10-100 mg/cm² to the wound-contacting surface of the matrix.
- 20 4. A hemostatic patch according to claim 2, wherein the epsilon aminocaproic acid is applied in an amount between 60-70 mg/cm² to the wound-contacting surface of the matrix.
- 25 5. A hemostatic patch according to claims 2 or 3, wherein the thrombin receptor activating peptide is present in an amount between of 0.25-100 mcg/cm².
6. A hemostatic patch according to claim 5, wherein the thrombin receptor activating peptide is present in amounts ranging between 1-100 mcg/cm².

7. A hemostatic patch according to claim 5, wherein the thrombin receptor activating peptide is present in an amount between 0.25-25 mcg/cm².

5 8. A hemostatic patch according to claim 5, wherein the thrombin receptor activating peptide is selected from the group of peptides with the amino acid sequences consisting of SFLLRNPNDKYEPF; SFLLRNPNDKYEP; SFLLRNPNDKYE; SFLLRNPNDKY; SFLLRNPNDK; SFLLRNPND; SFLLRNP; SFLLRNP; SFLLRN; SFLLR; SFLL; and SFL, and
10 the amidated forms thereof.

9. A hemostatic patch according to claim 5, wherein the thrombin receptor activating peptide is selected from the group or peptides consisting of TRAP 508-530 and TRAP 517-530.

15 10. A hemostatic patch according to claim 9, wherein a thrombin receptor activating peptide comprises the polynucleotide molecule TRAP 517-530.

11. A hemostatic patch according to claim 2, wherein said matrix is selected from the group
20 consisting of absorbable gelatin sponge, calcium alginate, calcium/sodium alginate, collagen, and oxidized regenerated cellulose.

12. A hemostatic patch according to claim 11, wherein the matrix is absorbable gelatin sponge.

25 13. A hemostatic patch according to claim 12, wherein said absorbable gelatin sponge is gelatin foam.

14. A hemostatic patch according to claim 13, wherein said gelatin foam is compressed to at least about one-half its original thickness.

15. A hemostatic patch according to claim 11, wherein the matrix is collagen.

16. A hemostatic patch according to claim 11, wherein the matrix is calcium alginate.

5 17. A hemostatic patch according to claim 5 wherein the matrix further comprises calcium ions in an amount effective for stimulating hemostasis.

18. A hemostatic patch according to claim 17, wherein calcium chloride is present in an amount
10 between 25-150 micrograms/cm² of the wound-contacting surface.

19. A hemostatic patch according to claim 18, wherein the amount of calcium chloride is between 50-100 micrograms/cm².

15 20. A hemostatic patch according to claims 2 or 3, which further comprises an amount of RGD or RGDS peptide effective for accelerating hemostasis.

21. A hemostatic patch according to claim 20, wherein RGD is present in an amount between 10-1000
20 mg/cm² of the wound-contacting surface.

22. A hemostatic patch according to claim 20, wherein said RGD peptide further comprises serine.

23. A hemostatic patch according to claim 5, wherein the matrix further comprises protamine sulfate
25 in an amount effective for neutralizing heparin present in the local environment of the patch.

24. A hemostatic patch according to claim 23, wherein protamine sulfate is present in an amount between 1-15 mg/cm² of the wound-contacting surface.

25. A hemostatic patch according to claim 24, wherein protamine sulfate is present in an amount between 2-5 mg/cm².

26. A hemostatic patch according to claim 2 which has a thickness of 2-10 millimeters.

27. A hemostatic patch according to claim 5 wherein the amounts of said hemostatic agents is sufficient to control hemorrhage in patients undergoing dicumarol therapy.

28. A hemostatic patch comprising a flat, flexible matrix that is biodegradable and absorbable, and to which is applied to a single wound-contacting surface along the length of said matrix, EACA in amounts of 10-100 mg/cm² and a thrombin receptor activating peptide in amounts of 0.25-100 mcg/cm², and optionally applied to said surface, one or more additives, including a calcium ion source, RGD peptide, RGDS peptide, protamine sulfate and buffer.

29. A hemostatic patch which is made by a process comprising the steps of:

- (a) applying an aqueous solution of EACA to a wound-contacting surface of a structural element that is a biodegradable matrix of compressed gelatin foam;
- (b) applying one or more thrombin receptor activating peptides to said wound-contacting surface; and
- (c) drying the resultant patch.

30. A method for effecting hemostasis in a bleeding wound, comprising applying a wound-contacting surface of a hemostatic patch according to claim 1 to a bleeding wound, and maintaining the patch in contact with the wound for a period of time sufficient to permit clotting to occur at the interface between the

hemostatic patch and the wound, and for bleeding to be substantially arrested.

31. A method according to claim 30, wherein said period of time is between 3 to 20 minutes.

5 32. A method according to claim 31, wherein said period of time is between 3 to 5 minutes.

33. A method according to claim 30, further comprising the step of moistening the patch with a sterile saline solution prior to applying the patch to
10 a wound.

34. A hemostatic bandage for applying to a wound on a patient, said bandage comprising a backing member fixedly secured to a patch according to claim 2, and at least one flap extending from said backing member
15 beyond said patch, said flap having applied thereto a medically acceptable adhesive suitable for adhering the bandage to the patient.

35. A hemostatic bandage according to claim 34,
20 wherein the epsilon aminocaproic acid is present in an amount between 10-100 mg/cm².

36. A hemostatic bandage according to claim 34 or 35, wherein the thrombin receptor activating peptide is present in an amount between of 0.25-100 mcg/cm².

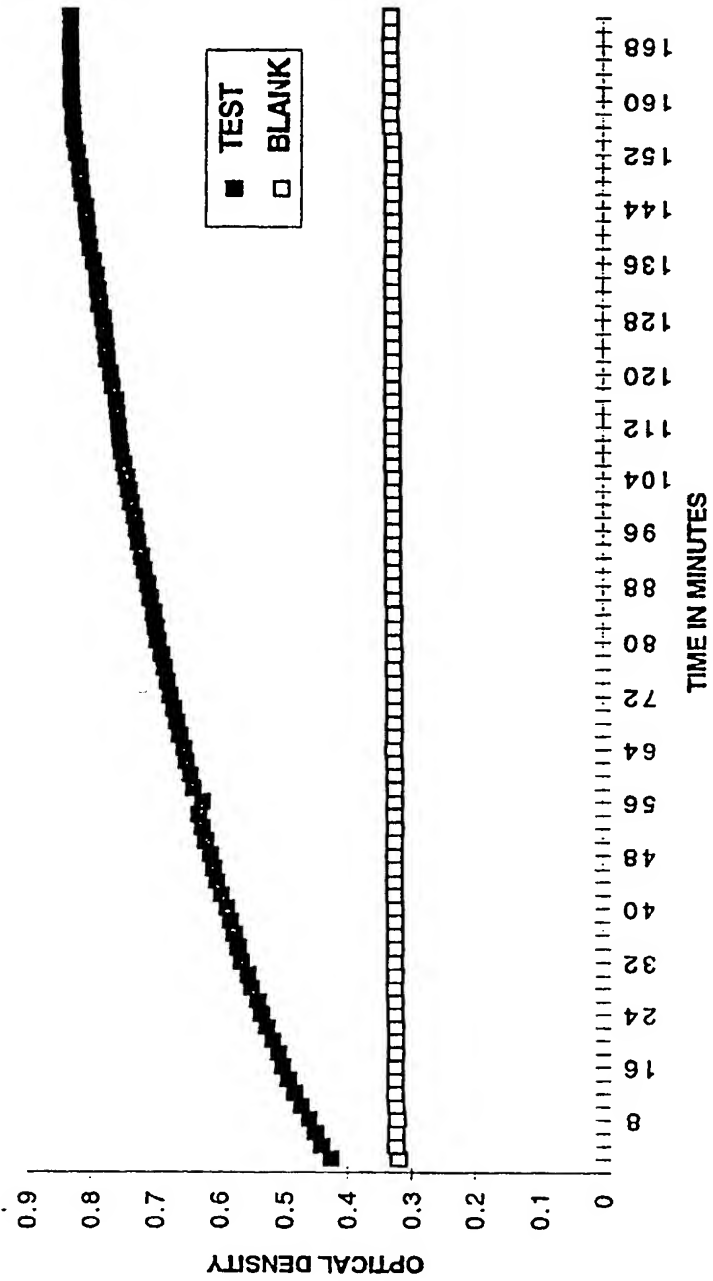
25 37. A kit comprising a sterile package containing a patch according to claim 2.

38. A kit according to claim 37, further comprising sterilized surgical instruments.

39. A kit according to claim 37, further
30 comprising at least one bandage.

1/9

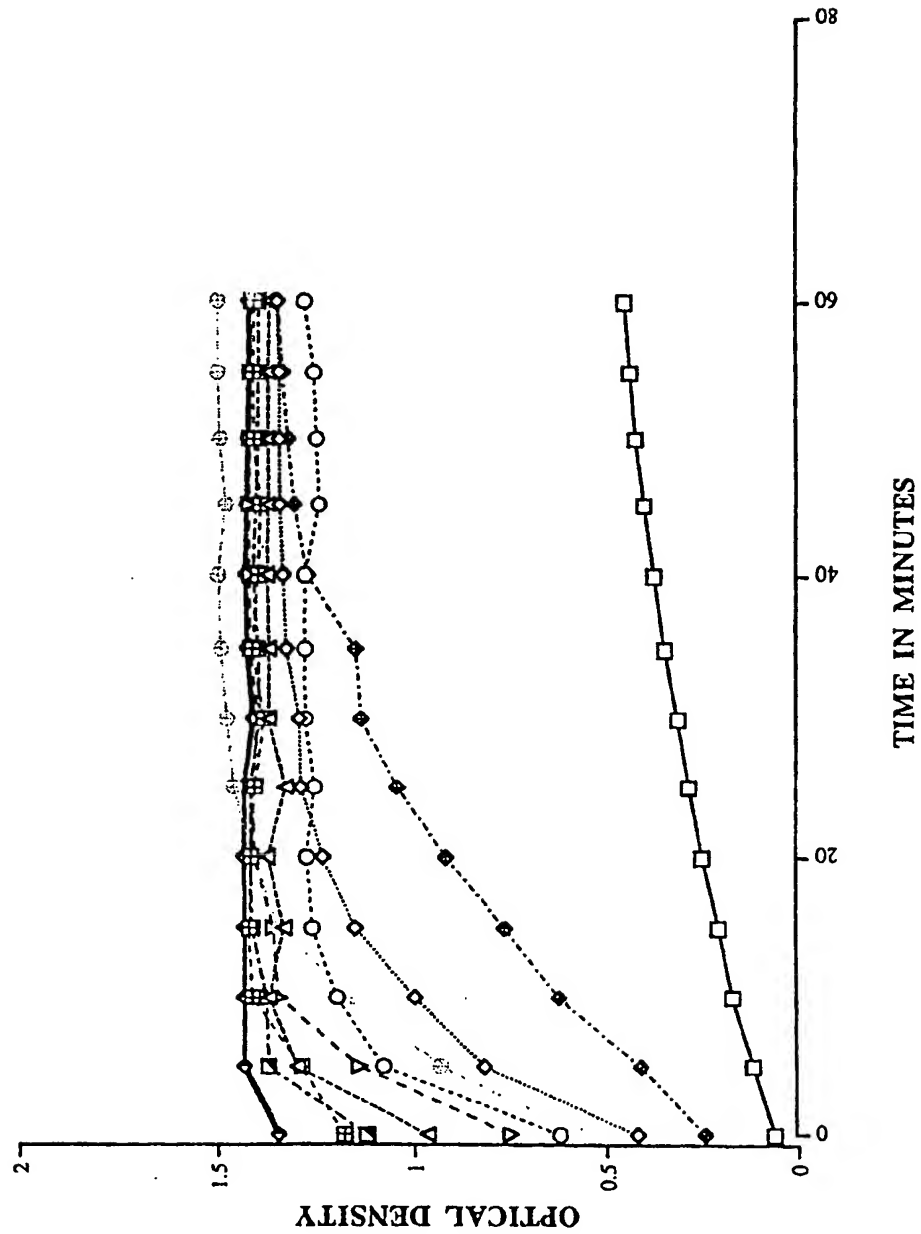
FIGURE 1



SUBSTITUTE SHEET (RULE 26)

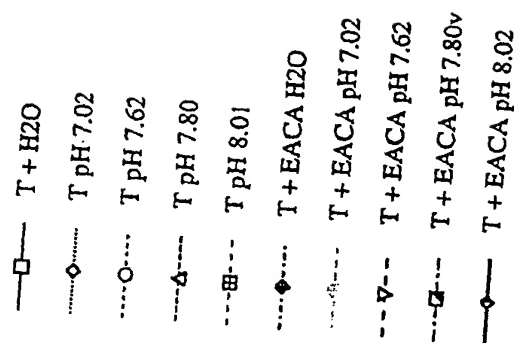
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FIGURE 2A



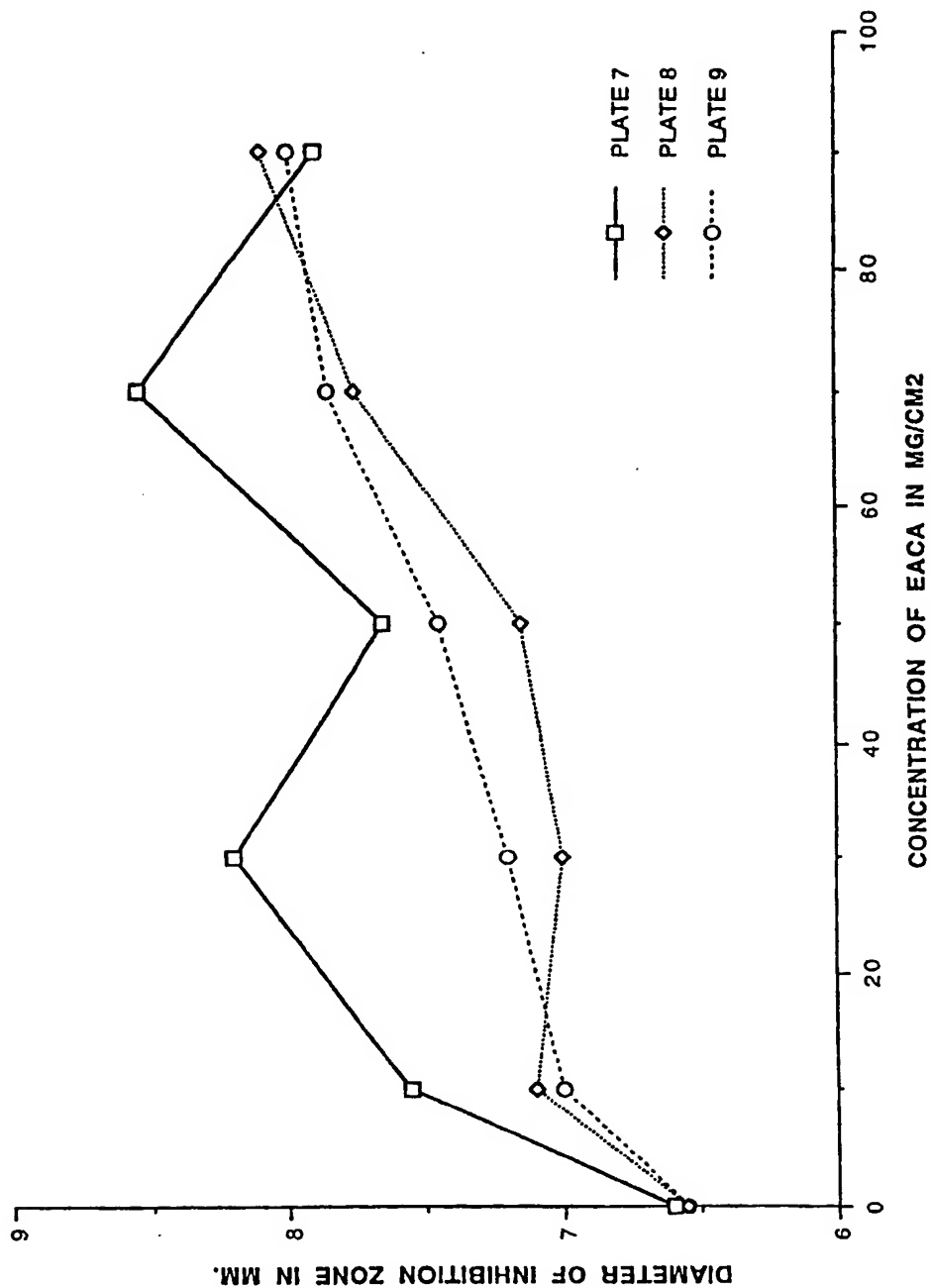
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FIGURE 2B



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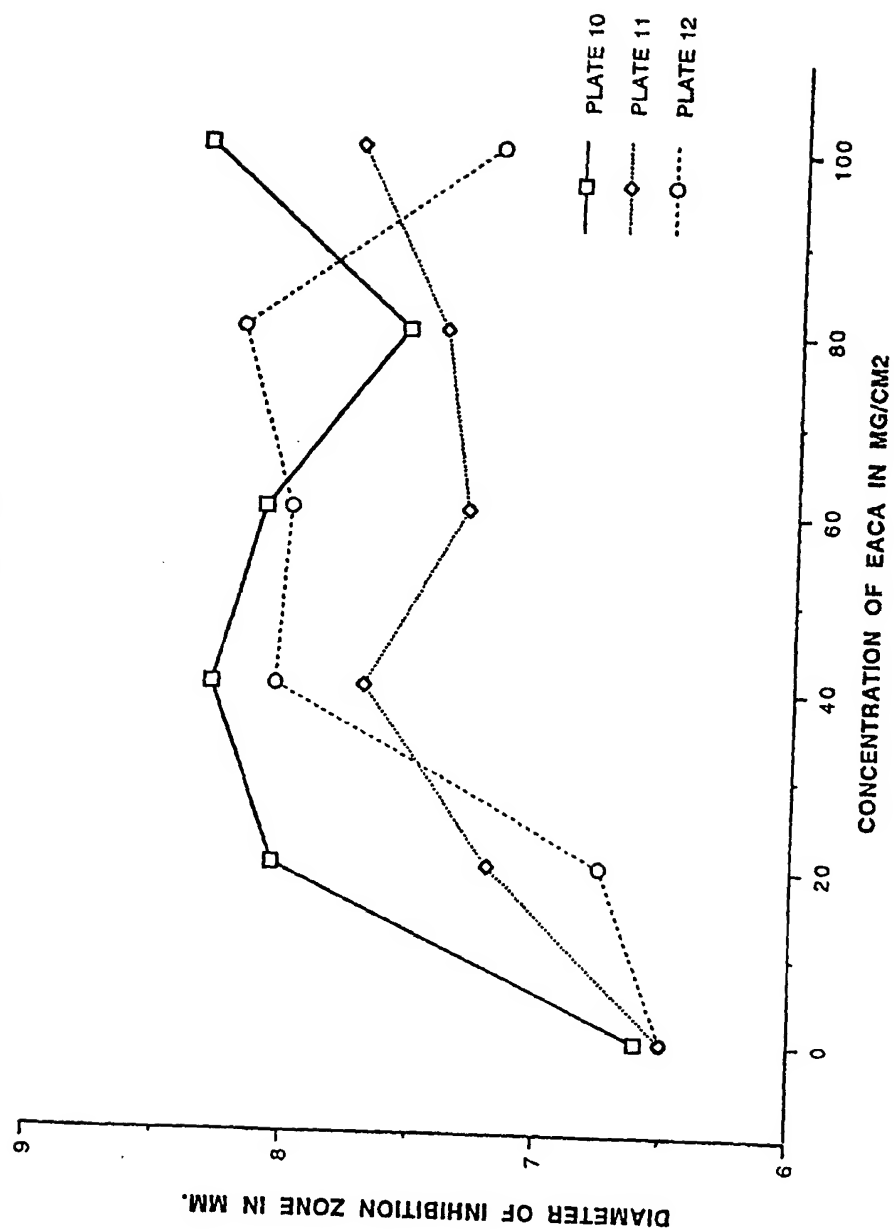
FIGURE 3A



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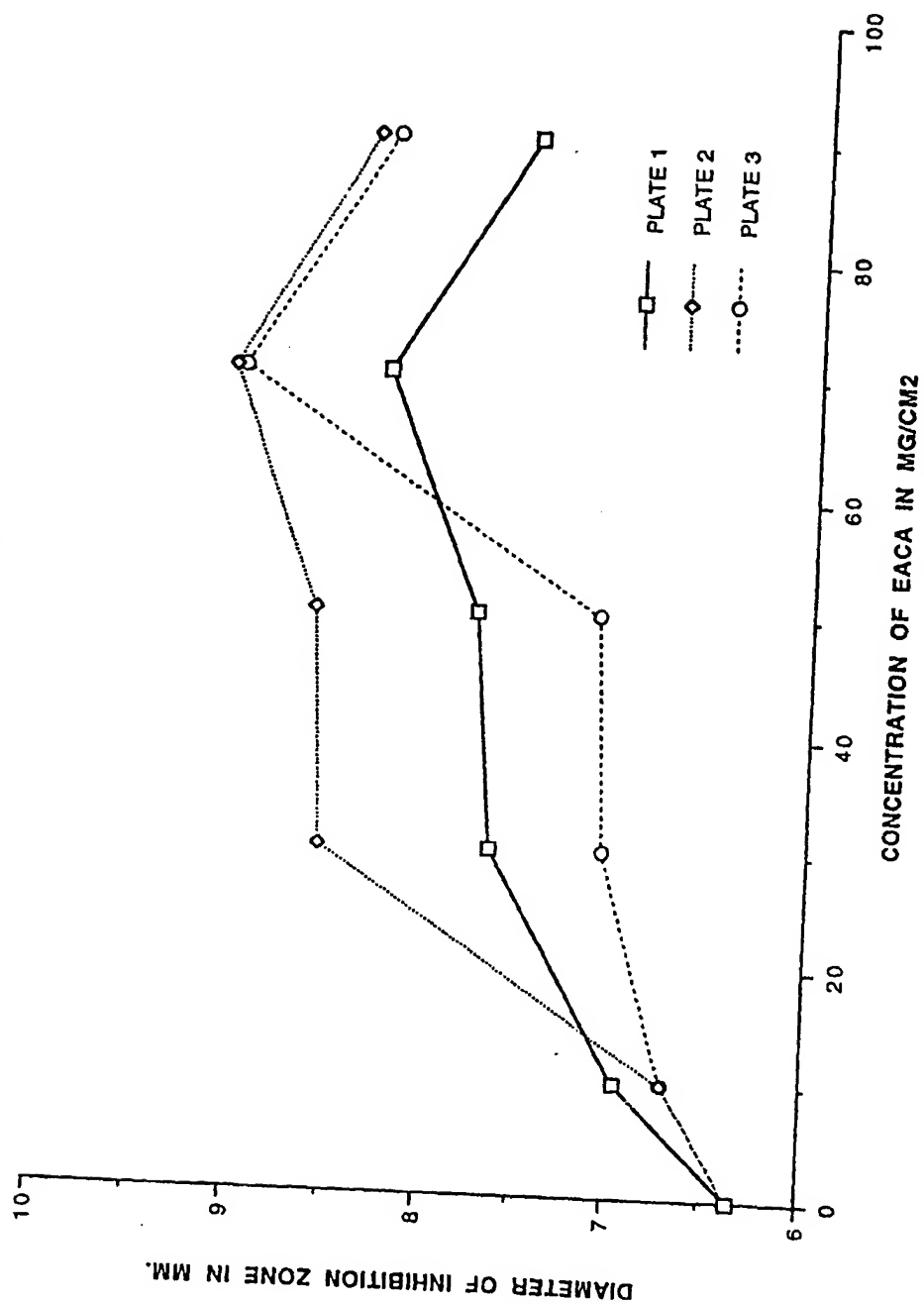
FIGURE 3B



SUBSTITUTE SHEET (RULE 26)

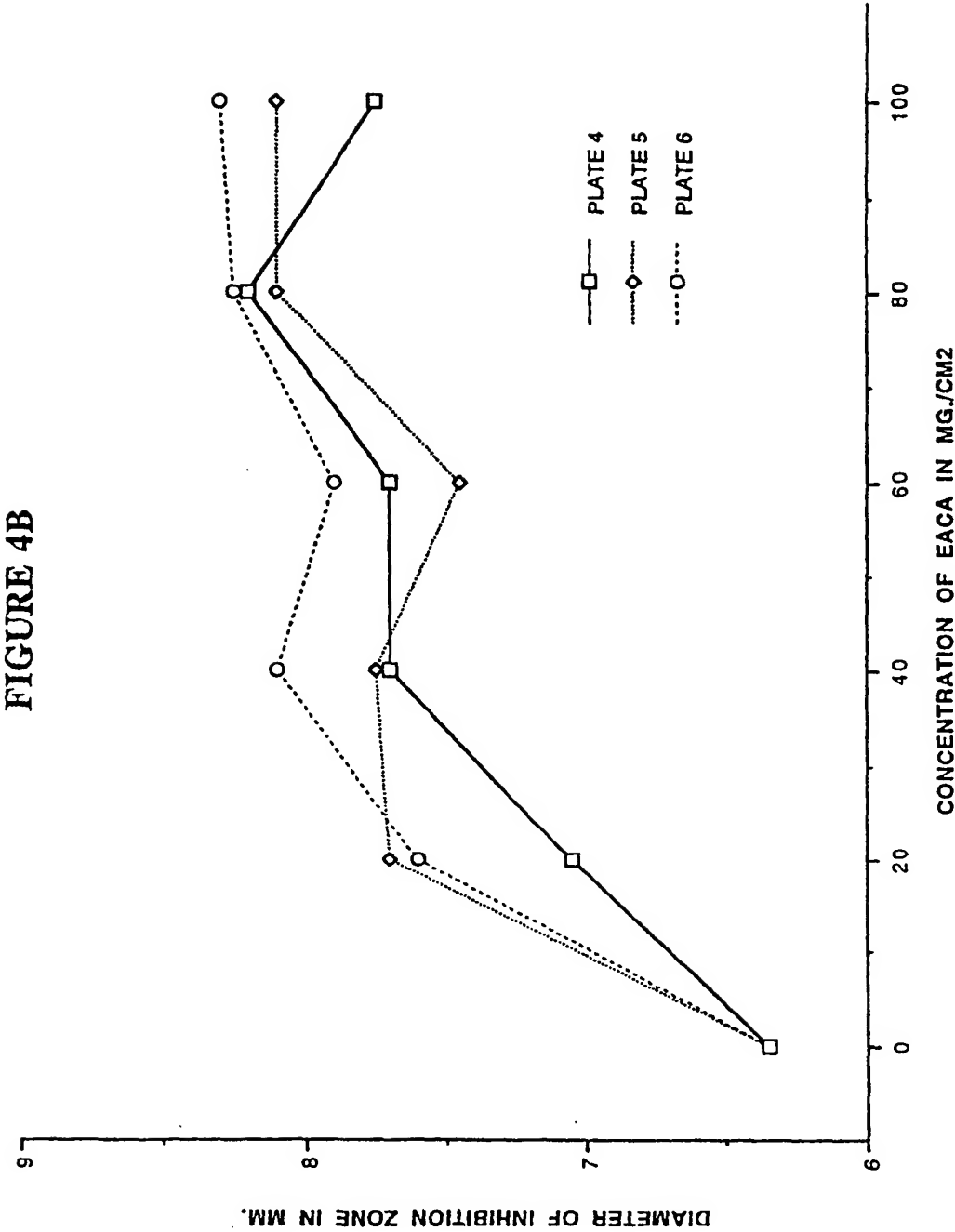
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FIGURE 4A



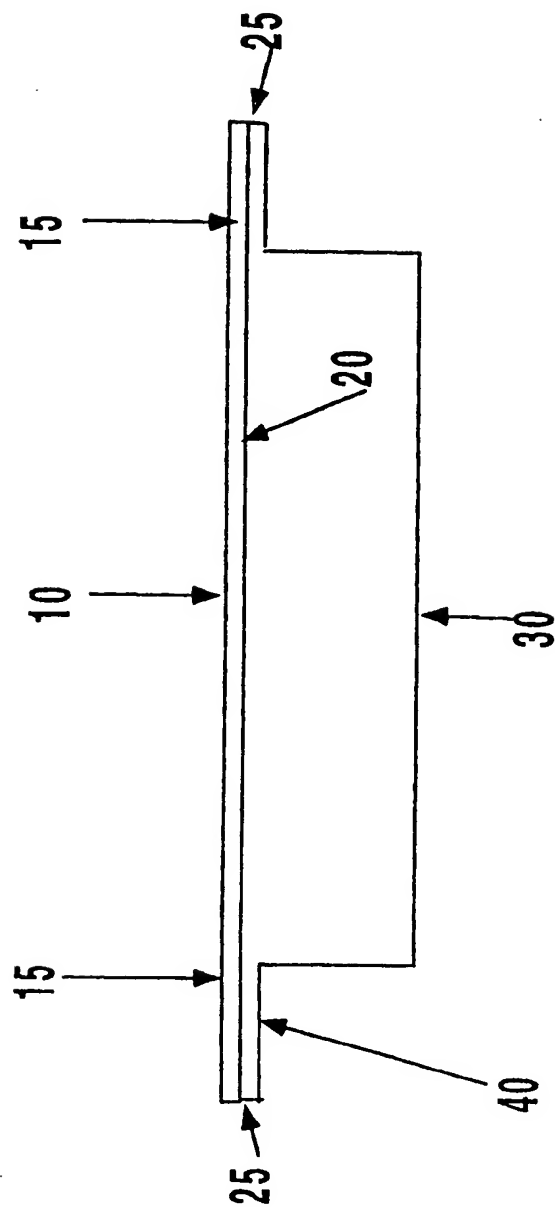
SUBSTITUTE SHEET (RULE 26)

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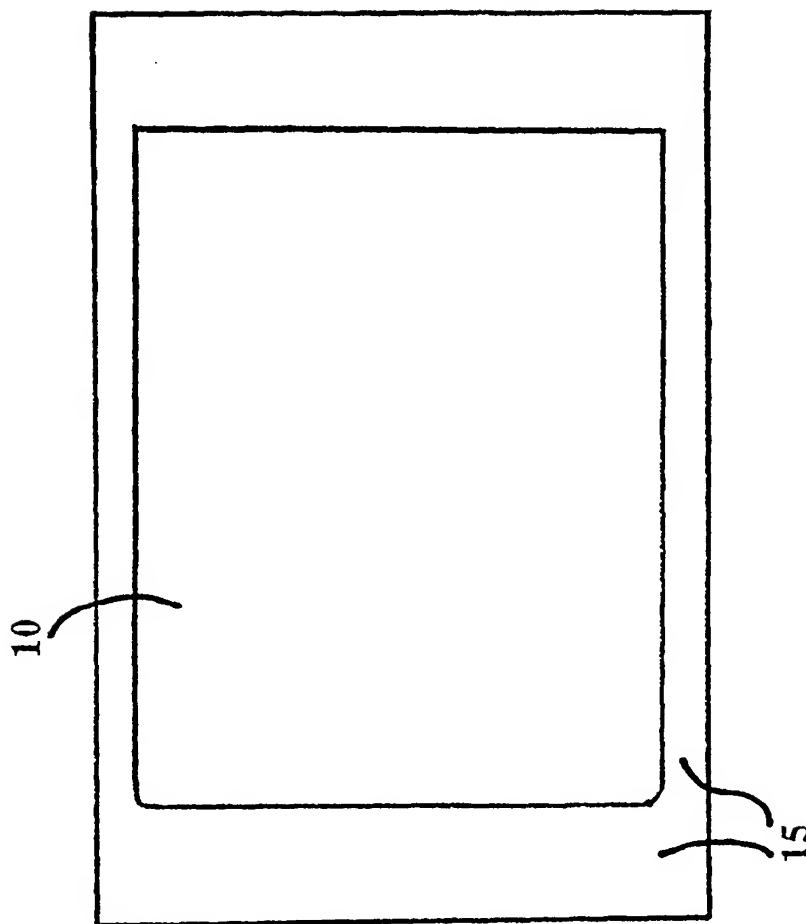
FIGURE 5A



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FIGURE 5B



SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06334

A. CLASSIFICATION OF SUBJECT MATTER														
IPC(6) : Please See Extra Sheet. US CL : Please See Extra Sheet. According to International Patent Classification (IPC) or to both national classification and IPC														
B. FIELDS SEARCHED														
Minimum documentation searched (classification system followed by classification symbols) U.S. : 424/443, 444, 445, 446, 447, 448, 449, 484, 485, 486, 487, 488; 514/2, 13, 14, 15, 16, 17, 18, 561; 530/331; 602/48, 49, 50														
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched														
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Extra Sheet.														
C. DOCUMENTS CONSIDERED TO BE RELEVANT														
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.												
A	US 4,427,650 A (M. STROETMANN) 24 January 1984 (24/01/84).	1-39												
Y	US 4,390,519 A (P. SAWYER) 28 June 1983 (28/06/83), column 4, lines 57-62.	34-36												
X --- Y	US 4,265,233 A (SUGITACHI ET AL) 05 May 1981 (05/05/81), abstract, column 2, line 1, column 8, lines 14-54, claims 3, 4, 17, 18, 20-22, 24.	1 - 7, 11, 12, 15, 17-19, 26-28, 30-32, 37-39 --- 8-10, 13, 14, 16, 20-25, 29, 33-36												
Y	US 4,749,689 A (MIYATA ET AL) 07 June 1988 (07/06/88), column 2, lines 36-41, column 2, line 63 -	1-7, 17-19, 23-28, 30-32, 37-												
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.														
<table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>*A* document defining the general state of the art which is not considered to be of particular relevance</td> <td>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>*E* earlier document published on or after the international filing date</td> <td>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>*Z* document number of the same patent family</td> </tr> <tr> <td>*O* document referring to an oral disclosure, use, exhibition or other means</td> <td></td> </tr> <tr> <td>*P* document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document number of the same patent family	*O* document referring to an oral disclosure, use, exhibition or other means		*P* document published prior to the international filing date but later than the priority date claimed	
* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention													
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone													
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art													
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document number of the same patent family													
O document referring to an oral disclosure, use, exhibition or other means														
P document published prior to the international filing date but later than the priority date claimed														
Date of the actual completion of the international search 10 JULY 1996		Date of mailing of the international search report 29 JUL 1996												
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer JEFFREY E. RUSSEL Telephone No. (703) 305-0196												

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06334

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,637,815 A (G. LEMOLE) 20 January 1987 (20/01/87), abstract.	1-7, 17-19, 23-28, 30-32, 37-39
A	US 4,442,655 A (M. STROETMANN) 17 April 1984 (17/04/84).	1-39
Y	US 5,256,766 A (S. COUGHLIN) 26 October 1993 (26/10/93), column 8, lines 30-36).	8-10
Y	US 5,041,380 A (RUOSLAHTI ET AL) 20 August 1991 (20/08/91), column 2, lines 19-48, column 6, line 44 - column 7, line 2.	20-22
A	US 4,891,359 A (SAFERSTEIN ET AL) 02 January 1990 (02/01/90).	1-39
Y	US 5,388,588 A (NABAI ET AL) 14 February 1995 (14/02/95), column 2, lines 8-11, column 3, lines 57-63, column 4, lines 35-37.	14,29
A	US 5,290,552 A (SIERRA ET AL) 01 March 1994 (01/03/1994).	1-39
Y	US 5,260,277 A (T. MCKENZIE) 09 November 1993 (09/11/93), column 4, line 62 - column 5, line 2.	20-22
Y	US 5,409,703 A (MCANALLEY ET AL) 25 April 1995 (25/04/95), column 2, lines 55-68.	13,14,29
Y,P	US 5,470,576 A (H. PATEL) 28 November 1995 (28/11/95), column 1, lines 16-61.	16
Y	CARNEY et al. Enhancement of Incisional Wound Healing and Neovascularization in Normal Rats by Thrombin and Synthetic Thrombin Receptor-activating Peptides. Journal of Clinical Investigation. May 1992, Volume 89, pages 1469-1477, especially the abstract, page 1470, column 1, fourth full paragraph.	8-10
Y	CORMACK et al. Acceleration of Soft Tissue Repair by a Thrombin-Derived Oligopeptide. Journal of Surgical Research. 1992, Volume 53, Number 2, pages 117-122, especially the abstract.	8-10

INTERNATIONAL SEARCH REPORT

Int. .onal application No.

PCT/US96/06334

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A61F 13/58; A61K 31/195, 38/06, 38/07, 38/08, 38/10, 38/16, 38/36; A61L 15/18, 15/20, 15/28, 15/32, 15/44

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/444, 445, 446, 447, 448, 449, 484, 485, 486, 488; 514/2, 13, 14, 15, 16, 17, 18, 561; 602/48, 49, 50

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG, MEDLINE

search terms: epsilon-aminocaproic acid, epsilon-aminohexanoic acid, thrombin, bandage, patch, protamine sulfate, gelatin, foam, calcium alginate, RGD, RGDS